553277

STIC-ILL

From: Davis, Minh-Tam
Sent: Wednesday, August 31, 2005 11:48 AM
To: STIC-ILL
Subject: REPRINT REQUEST FOR 10/069973

Adonis
BioTech MAIN
Vol NO NO3
Dupl Request
8131

EC

Mitochondrial permeability transition in CNS trauma: Cause or effect of neuronal cell death?
AUTHOR: Sullivan P G (Reprint); Rabchevsky A G; Waldmeier P C; Springer J E
AUTHOR ADDRESS: Spinal Cord and Brain Injury Res Ctr, Univ Kentucky, 240 HSRB, Lexington, KY, 40536, USA**USA
AUTHOR E-MAI

2) **Long-term follow-up and complications after cardiac transplantation.**
Conrad S A; Chhabra A; Vay D
Willis Knighton-LSU Medical Center Heart and Lung Transplantation Center in Shreveport.
Journal of the Louisiana State Medical Society - official organ of the Louisiana State Medical Society (UNITED STATES) May **1993** , 145 (5) p217-20, 223-5, ISSN 0024-6921 Journal Code: 7505618
Publishing Model Print

3) **Prevention of cardiac hypertrophy in mice by calcineurin inhibition.**
Sussman M A; Lim H W; Gude N; Taigen T; Olson E N; Robbins J; Colbert M C ; Gualberto A; Wieczorek D F; Molkentin J D
Division of Molecular Cardiovascular Biology, Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA.
Science (UNITED STATES) Sep 11 **1998** , 281 (5383) p1690-3, ISSN 0036-8075 Journal Code: 0404511

THANK YOU
MINH TAM DAVIS
ART UNIT 1642, ROOM 3A24, MB 3C18
272-0830

The following term was not found and ignored: perability.
See Details.
Did you mean: *sullivan[auth] AND mitochondrial **permeability** transition in cns trauma* (1 items)

1: Sullivan PG, Rabchevsky AG, Waldmeier PC, Springer JE. Related Articles, Links

Mitochondrial permeability transition in CNS trauma: cause or effect of neuronal cell death?
J Neurosci Res. 2005 Jan 1-15;79(1-2):231-9. Review.
PMID: 15573402 [PubMed - indexed for MEDLINE]

Journal of Neuroscience Research 79:231–239 (2005)

Mitochondrial Permeability Transition in CNS Trauma: Cause or Effect of Neuronal Cell Death?

P.G. Sullivan,[1,2*] A.G. Rabchevsky,[1,3] P.C. Waldmeier,[4] and J.E. Springer[1,2]
[1]Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, Kentucky
[2]Department of Anatomy and Neurobiology, University of Kentucky, Lexington, Kentucky
[3]Department of Physiology, University of Kentucky, Lexington, Kentucky
[4]Nervous System Research, Novartis Pharma Ltd., Basel, Switzerland

Experimental traumatic brain injury (TBI) and spinal cord injury (SCI) result in a rapid and significant necrosis of neuronal tissue at the site of injury. In the ensuing hours and days, secondary injury exacerbates the primary damage, resulting in significant neurologic dysfunction. It is believed that alterations in excitatory amino acids (EAA), increased reactive oxygen species (ROS), and the disruption of Ca^{2+} homeostasis are major factors contributing to the ensuing neuropathology. Mitochondria serve as the powerhouse of the cell by maintaining ratios of ATP:ADP that thermodynamically favor the hydrolysis of ATP to ADP + P_i, yet a byproduct of this process is the generation of ROS. Proton-pumping by components of the electron transport system (ETS) generates a membrane potential ($\Delta\Psi$) that can then be used to phosphorylate ADP or sequester Ca^{2+} out of the cytosol into the mitochondrial matrix. This allows mitochondria to act as cellular Ca^{2+} sinks and to be in phase with changes in cytosolic Ca^{2+} levels. Under extreme loads of Ca^{2+}, however, opening of the mitochondrial permeability transition pore (mPTP) results in the extrusion of mitochondrial Ca^{2+} and other high- and low-molecular weight components. This catastrophic event discharges $\Delta\Psi$ and uncouples the ETS from ATP production. Cyclosporin A (CsA), a potent immunosuppressive drug, inhibits mitochondrial permeability transition (mPT) by binding to matrix cyclophilin D and blocking its binding to the adenine nucleotide translocator. Peripherally administered CsA attenuates mitochondrial dysfunction and neuronal damage in an experimental rodent model of TBI, in a dose-dependent manner. The underlying mechanism of neuroprotection afforded by CsA is most likely via interaction with the mPTP because the immunosuppressant FK506, which has no effect on the mPT, was not neuroprotective. When CsA was administrated after experimental SCI at the same dosage and regimen used TBI paradigms, however, it had no beneficial neuroprotective effects. This review takes a comprehensive and critical look at the evidence supporting the role for mPT in central nervous system (CNS) trauma and highlights the differential responses of CNS mitochondria to mPT induction and the implications this has for therapeutically targeting the mPT in TBI and SCI.

Key words: neuronal cell death; reactive oxygen species; mitochondrial permeability transition pore; mitochondrial dysfunction; traumatic brain injury; spinal cord injury

Mitochondria are uniquely poised to play a pivotal role in neuronal cell survival or death after central nervous system (CNS) injury because they are regulators of both energy metabolism and apoptotic pathways (Fiskum, 2000; Wieloch, 2001; Friberg and Wieloch, 2002). Maintaining mitochondrial homeostasis and bioenergetics in neurons is even more critical due to their almost complete dependence on mitochondrial-derived ATP (Budd and Nicholls, 1998; Sullivan et al., 1998, 2000a; Nicholls and Budd, 2000). Mitochondria also serve as high capacity Ca^{2+} sinks, which allows them to stay in tune with changes in cytosolic Ca^{2+} loads and aid in maintaining cellular Ca^{2+} homeostasis that is required for normal neuronal function (Ichas and Mazat, 1998; Rizzuto et al., 1999, 2000). Conversely, excessive Ca^{2+} uptake into mitochondria has been shown to increase reactive oxygen species (ROS) production, inhibit ATP synthesis, release cytochrome c, and induce mitochondrial permeability transitions (Sullivan et al., 1999b, 2000a, 2004; Jiang et al., 2001; Brustovetsky et al., 2002, 2003).

The mitochondrial permeability transition (mPT) is defined as the sudden increase of inner mitochondrial membrane permeability to solutes of molecular mass less than 1,500 Daltons (Gebicki and Hunter, 1964; Hunter

*Correspondence to: Patrick Sullivan, PhD, University of Kentucky, Spinal Cord and Brain Injury Research Center, 240 HSRB, Lexington, KY 40536-0305. E-mail: PatSull@uky.edu

Received 9 July 2004; Revised 29 July 2004; Accepted 5 August 2004

Published online 30 November 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20292

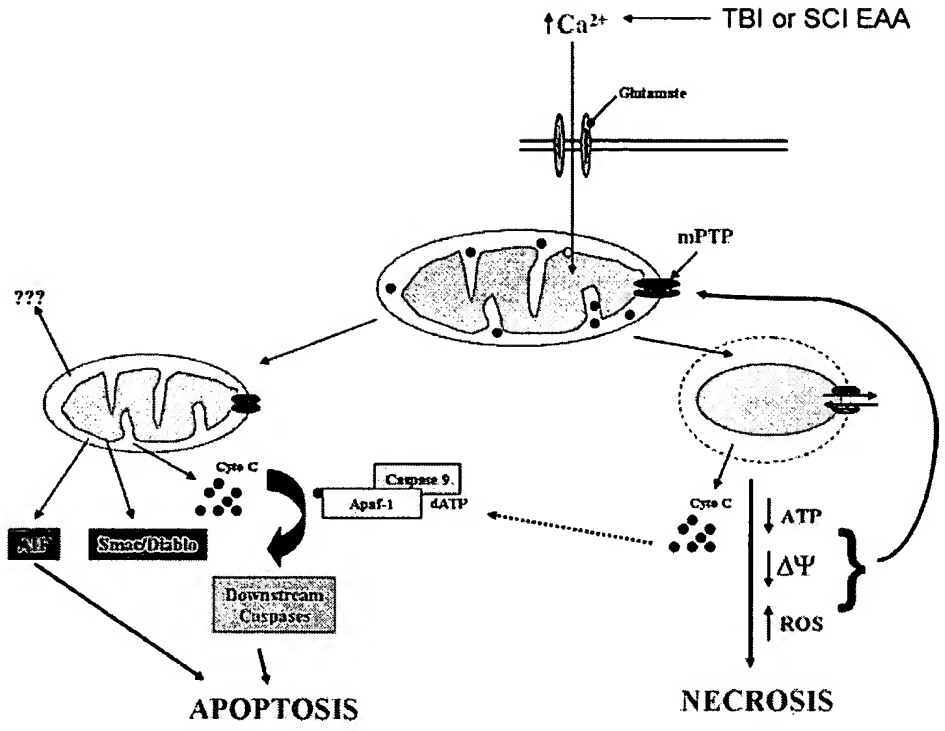

Fig. 1. Highly simplified schematic diagram illustrating the pivotal role of mitochondria in apoptotic and necrotic neuronal cell death after CNS injury. After traumatic brain injury (TBI) or spinal cord injury (SCI), excitatory amino acid (EAA) release increases Ca^{2+} influx into neurons. Mitochondria sequester the Ca^{2+} in an effort to maintain cytosolic Ca^{2+} homeostasis. Excessive mitochondrial Ca^{2+} cycling/loading can result in mitochondrial permeability that can be mediated by the mitochondrial permeability transition pore (mPTP) opening or via increased permeability of the outer membrane without mPTP opening. In either case proapoptotic proteins such as Smac/Diablo, apoptosis-inducing factor (AIF), and cytochrome c (Cyto C) can be released from the mitochondria leading to activation of downstream caspases and apoptosis. Ca^{2+}-induced mPT results in increased conductance of the inner membrane, mitochondrial swelling, loss of ATP production due to decreased membrane potential ($\Delta\Psi$), and increased reactive oxygen species (ROS) production. These events promote mPT and can lead to necrosis depending the extent and severity of the insult/injury.

et al., 1964a,b; Bernardi et al., 1994; Bernardi, 1996; Nicolli et al., 1996). Strong evidence now exists that the mPT is due to the opening of a nonselective megachannel (estimated to be 2–3 nm in diameter) referred to as the mitochondrial permeability transition pore (mPTP) (Szabo and Zoratti, 1991, 1992; Szabo et al., 1992). Because the chemiosmotic theory is based on the inner membrane being impermeable to solutes that are not specifically transported, mPT would collapse the mitochondrial membrane potential ($\Delta\Psi$) and uncouple the electron transport system from the production of ATP. Additionally mPT results in mitochondrial swelling and can lead to the release of proapoptotic proteins (Fig. 1). Importantly, Ca^{2+}, P_i, oxidative stress, and low inner membrane potentials promote the onset of mPT, whereas cyclosporin A (CsA), Mg^{2+}, ADP, and the existence of a high membrane potential oppose the onset (Bernardi, 1996; Hansson et al., 2004).

The molecular identity of the mPTP has not yet been established, but the current accepted model points to several well-known membrane components, as well as proteins found in the cytosol, the intermembrane space, and the matrix of mitochondria. The adenine nucleotide translocase (ANT) has been proposed to be a core component of the mPTP (Halestrap and Davidson, 1990; Halestrap et al., 1997; Woodfield et al., 1998; McStay et al., 2002; Halestrap and Brennerb, 2003); however, its role is controversial (Clarke et al., 2002; Halestrap et al., 2002; Waldmeier et al., 2003; Kokoszka et al., 2004). ANT is located in the inner mitochondrial membrane and may play a central role in the formation of the pore by establishing complexes with outer mitochondrial membrane proteins such as Porin (VDAC) to create contact sites that stabilize or enlarge the pore complex (Bernardi et al., 1994; Zoratti and Szabo, 1994; Beutner et al., 1998). When reconstituted, these ANT-VDAC complexes have been shown to bind several other proteins that are known to modulate the mPTP, including the cytosolic protein hexokinase and creatine kinase, which is found in the mitochondrial intermembrane space (Beutner et al., 1996, 1998). Additionally, Bcl-2 antiapoptotic proteins and proapoptotic proteins such as Bax bind the ANT-VDAC complex (Marzo et al., 1998; Brenner et al., 2000). The ANT may also be the site of action for the most potent

inhibitor of the mPTP, CsA via its interaction with cyclophilin-D (CyP-D).

CsA is an undecapeptide of fungal origin that is used clinically as an immunosuppressive agent. CsA immunosuppressive properties stem from its inhibition of calcineurin. CyP-D is a matrix specific peptidyl-prolyl *cis–trans* isomerase that translocates from the matrix to the mPTP where it is proposed to interact with the ANT and induce pore formation (Halestrap and Davidson, 1990; Halestrap et al., 1997; Clarke et al., 2002; Halestrap et al., 2002; Waldmeier et al., 2003). CsA or its nonimmunosuppressive analog *N*-methyl-Val-4-CsA inhibits the mPT by blocking the interaction of CyP-D with the ANT, thus blocking a conformational change in ANT (Broekemeier et al., 1989; Szabo and Zoratti, 1991; Connern and Halestrap, 1994; Broekemeier and Pfeiffer, 1995). Other modulators of the ANT and the mPTP include bongkrekic acid and atractyloside, which inhibit and induce, respectively, the induction of the mPT (Brustovetsky and Dubinsky, 2000; Jiang et al., 2001; Sullivan et al., 2004).

Although an exact physiologic role for the mPT has not yet been established, several studies have implicated a role for mitochondrial Ca^{2+} cycling after the induction of long-term potentiation (Stanton and Schanne, 1986; Bindokas et al., 1998) and that CsA blocks both long-term potentiation and long-term depression (Lu et al., 1996; Kamal et al., 1999). It is apparent, however, that prolonged opening of the mPTP would inactivate mitochondria rapidly and result in cell death. This would indicate a clear role for the mPT in neuropathology. The mPT may be of particular importance after traumatic brain injury (TBI) and spinal cord injury (SCI) because many pro-mPT events are known to occur as a result of these injuries.

MITOCHONDRIAL DYSFUNCTION IN TBI AND SCI

Several pathophysiologic events after CNS trauma contribute to neuronal damage and death, including glutamate-mediated excitotoxicity, ROS formation, and subsequent lipid peroxidation (Faden et al., 1989; Braughler and Hall, 1992; Azbill et al., 1997; Sullivan et al., 1998, 1999b). Mitochondrial ROS generation has been shown to increase after glutamate exposure (Coyle and Puttfarcken, 1993; Dugan et al., 1995) and has been linked directly to Ca^{2+} influx into the mitochondria (Reynolds and Hastings, 1995; Prehn, 1998; Scanlon and Reynolds, 1998; Sengpiel et al., 1998; Carriedo et al., 2000; Liang et al., 2000). Furthermore, ROS production and lipid peroxidation increase after experimental TBI (Sullivan et al., 1998) and SCI (Braughler and Hall, 1992; Hall et al., 1992; Azbill et al., 1997), whereas interventions that reduce ROS production at the mitochondrial level have been shown to be neuroprotective in several paradigms (Keller et al., 1998; Sullivan et al., 1999a; Liang et al., 2000).

It has been established that Ca^{2+}-induced mitochondrial dysfunction occurs acutely after TBI (Sullivan et al., 1998, 1999b, 2000a; Lifshitz et al., 2003). Increased mitochondrial Ca^{2+} loading has also been demonstrated to occur in neurosynaptosomes isolated from injured brain and spinal cord (Azbill et al., 1997; Sullivan et al., 1999b, 2000a). As illustrated in Figure 2, TBI and SCI both result in reduced mitochondrial bioenergetics demonstrated by altered oxygen consumption during different states of mitochondrial respiration. This loss of mitochondrial electron transport system (ETS) capacity would be expected to reduce $\Delta\Psi$, which has been documented in CNS injury (Azbill et al., 1997; Sullivan et al., 1999b, 2000a).

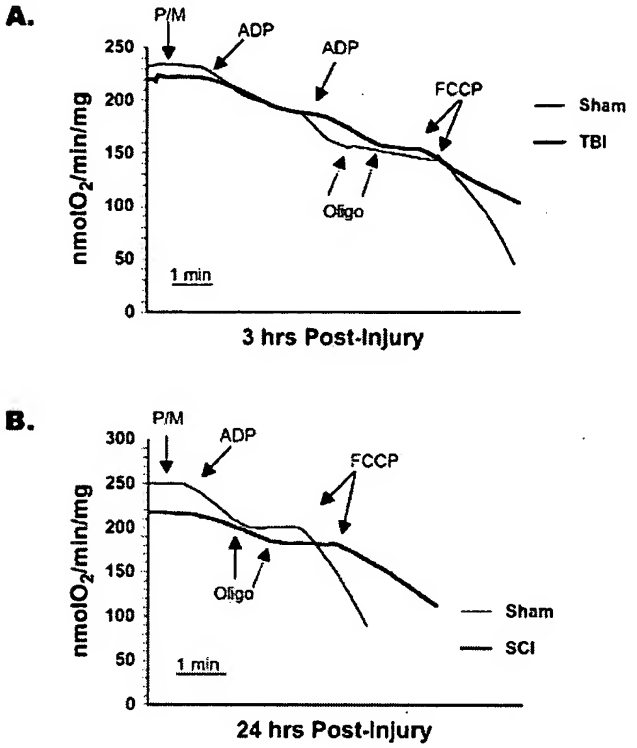

Fig. 2. Traumatic brain injury (TBI) or spinal cord injury (SCI) alter synaptic mitochondrial bioenergetics. Synaptic mitochondria isolated 3 hr post-injury from the injured cortex of TBI animals demonstrate a reduction in respiratory control ratio (rate of respiration in the presence of ADP/rate of respiration in the absence of ADP), a loss of electron transport system capacity (rate of respiration in the presence of the uncoupler FCCP), and a reduction in oxidative phosphorylation evident by reduced O_2 consumption after the addition of ADP (**A**). Synaptic mitochondria isolated from the spinal cord of SCI animals demonstrated a similar loss of mitochondria homeostasis even at 24 hr post-injury (**B**).

Because these post-injury events (increased mitochondrial Ca^{2+}, increased oxidative damage, and decreased mitochondrial membrane potentials) are known to be associated with mPT induction, it is not surprising that TBI reduces the threshold for Ca^{2+}-induced mPT in isolated mitochondria after injury (Sullivan et al., 1999b, 2000a; Lifshitz et al., 2003). Another consequence of mPT that has been documented after TBI and SCI is the redistribution of cytochrome c from mitochondria into the cytosol and activation of downstream caspases (Springer

et al., 1999; Lewen et al., 2001; Sullivan et al., 2002). These data indicate the prominent role that mitochondria and possibly the mPT play in neuronal cell survival and death after CNS injury.

THE MPT AS A THERAPEUTIC TARGET IN TBI AND SCI

It has been demonstrated that a pre-injury intrathecal bolus of CsA (20 mg/kg) reduced diffuse axonal injury and Ca^{2+}-induced cytoskeletal damage after TBI in rats (Okonkwo et al., 1999; Okonkwo and Povlishock, 1999). The clinically relevant question of CsA efficacy post-injury was then tested in two different strains of mice and in rats by Scheff and Sullivan (1999). In this study, CsA was found to significantly reduce cortical damage (50%) after TBI when administered intraperitoneally at 15 min and 24 hr post-injury. It was also shown in this study that there seemed to be a biphasic dose response for the neuroprotective benefits of CsA with the greatest protection afforded by the lowest dose (20 mg/kg, intraperitoneally [i.p.]) (Scheff and Sullivan, 1999).

It was also demonstrated that the immunosuppressive properties of CsA were most likely not responsible for the neuroprotection because the more potent immunosuppressor FK506 afforded no neuroprotection in this paradigm (Scheff and Sullivan, 1999). Both FK506 and CsA inhibit calcineurin, which reduces T-cell activation. In a follow-up study, mitochondrial function was assessed directly after TBI (Sullivan et al., 1999b), and results suggested that the neuroprotective properties of CsA were mediated through modulation of the mPTP and maintenance of mitochondrial homeostasis after TBI. FK506, however, has been shown to reduce diffuse axonal damage when employed in the less severe impact-acceleration model of TBI (Singleton et al., 2001; Suehiro et al., 2001). It was demonstrated that administration of CsA (20 mg/kg; i.p) 15 min post-injury significantly attenuated mitochondrial dysfunction measured using several biochemical assays of mitochondria integrity and bioenergetics (Fig. 3).

Although CsA is FDA approved and used routinely as an immunosuppressor in clinical settings, it has been shown to have significant dose-dependent neurologic effects, including seizures and cell death (Berden et al., 1985; de Groen et al., 1987; Walker and Brochstein, 1988; Famiglio et al., 1989; Kahan, 1989). It was therefore critical to determine the complete dose curve for CsA neuroprotection after TBI. To address this question, injured animals were administered various dosages of CsA or vehicle 15 min post-injury with a subsequent injection 24 hr later. The most advantageous CsA therapy was a 20 mg/kg bolus i.p. injection initiated 15 min post-injury (Sullivan et al., 2000b). Although other doses of CsA (ranging from 40 mg/kg to 1 mg/kg) significantly reduced cortical damage compared to vehicle-treated animals, the 20-mg/kg dosage afforded the greatest neuroprotection.

The extent of the post-injury therapeutic window was also established in this study by administering the optimal dose of CsA at various times after injury. The results demonstrated that CsA therapy was effective when initiated at 15 min, 1 hr, or 24 hr post-injury. Paradoxically, the efficacy was lost completely if therapy was initiated at 6 hr post-injury. The fact that delaying the onset of treatment until 24 hr post-injury was as effective as initiation at 1 hr illustrates that the mechanisms responsible for tissue destruction were active and amenable to pharmacologic manipulation at least 24 hr after the initial injury. The lack of neuroprotection by CsA if administration is delayed for 6 hr post-injury may highlight the role the blood–brain barrier (BBB) in designing CNS injury therapeutics (Baldwin et al., 1996). CsA does not freely cross the intact BBB (Shiga et al., 1992; Uchino et al., 1995, 1998), so after TBI this allows targeting of CsA to the injured tissue. Any fluctuations in the permeability (i.e., closure) of the BBB post-injury, however, would also hinder CsA uptake.

Because the time frame of secondary injury resulting from TBI ranges from minutes to days, and given the dynamic state of BBB post-injury, continuous dosing of CsA could be very advantageous. To address this question, animals were administrated an i.p. bolus of CsA or vehicle 15 min post-injury, then delivered CsA continuously for 7 days post-injury. All animals receiving CsA demonstrated a significant reduction in lesion volume, with the highest dose offering the most neuroprotection (74% reduction in lesion volume) (Sullivan et al., 2000c). These data highlight the need for designing multiple dosing regimens after TBI. Although all animals receiving CsA were significantly better than were vehicle-treated controls, injured animals receiving only a single bolus injection were afforded the least protection.

These results, coupled with the commonality of mitochondrial dysfunction demonstrated in both TBI and SCI, would indicate that CsA should offer some degree of neuroprotection in the injured spinal cord. Surprisingly, using the optimal dosage and regimen used in our TBI paradigms, CsA had no beneficial effects on histologic sparing after SCI (Rabchevsky et al., 2001). We hypothesized that this differential response to CsA treatment could be due to fundamental differences in brain and spinal cord mitochondria. Differences in the thresholds and responses of mitochondria to Ca^{2+}-induced mPT isolated from different regions of the brain are in fact well documented (Friberg et al., 1999; Brustovetsky et al., 2003). Accordingly, our group has reported recently that mitochondria isolated from the brain and spinal cord are significantly divergent (Sullivan et al., 2004).

Specifically, we found significant differences in mitochondrial lipid peroxidation, in situ ROS production, mtDNA oxidation, complex I enzyme activity, and NADH-linked respiration in the spinal cord compared to the cortex. Additionally, specific mitochondrial mRNA levels were reduced significantly in segments of spinal cord compared to that in the cortex. As illustrated in Figure 4, spinal cord mitochondria undergo mPT at significantly lower concentrations of Ca^{2+} than do cortical mitochondria. Increased oxidative stress, however, was sufficient to reduce the threshold for mPT in cortical mitochondrial.

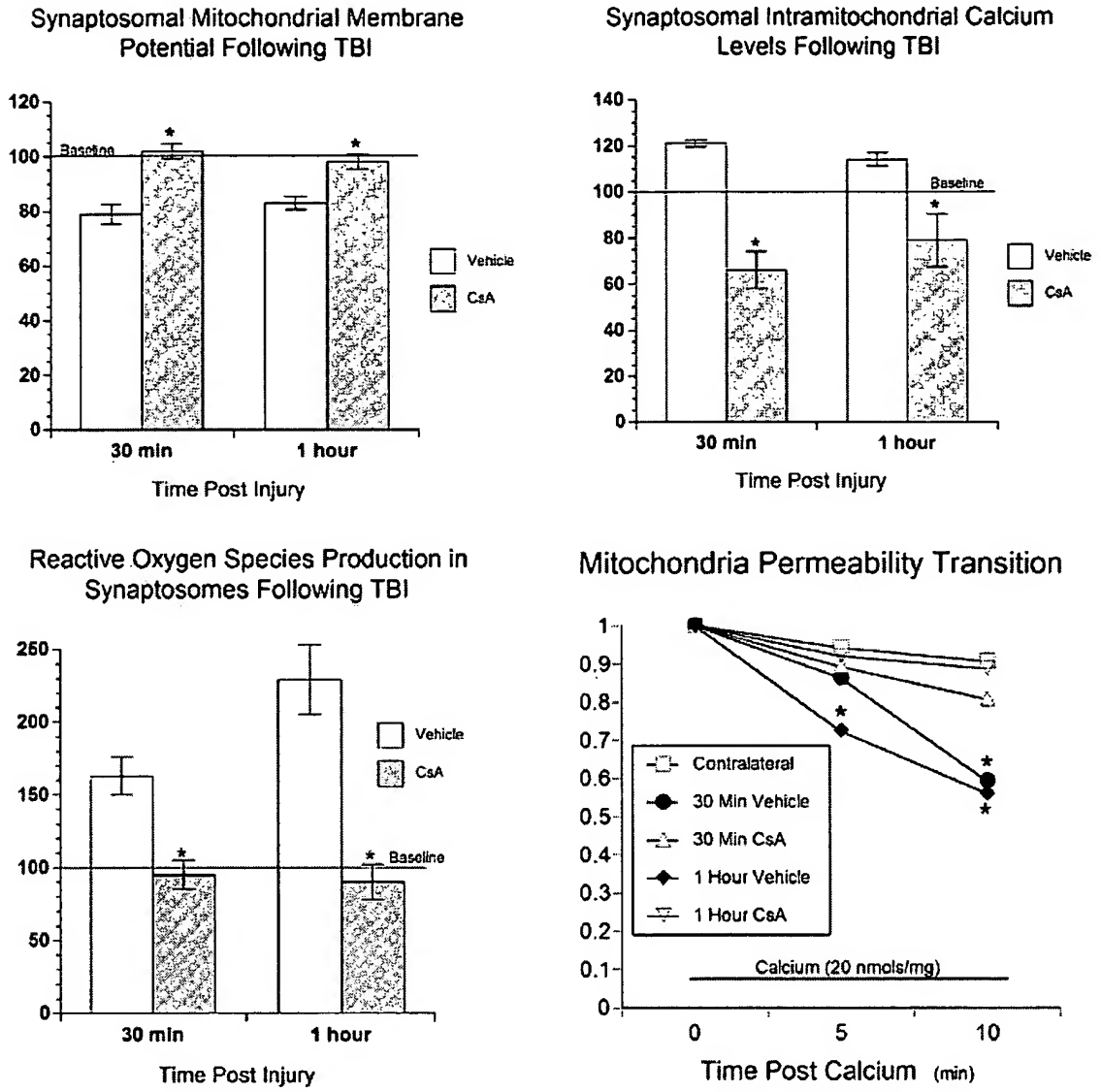

Fig. 3. Cyclosporin A (CsA) improves mitochondrial function after traumatic brain injury (TBI). CsA or vehicle was administered 15 min post-injury and mitochondria isolated from the ipsilateral (injured) or contralateral cortex at 30 min or 1 hr post-injury. Several parameters of mitochondrial function were assessed including membrane potential (upper left), Ca^{2+} loads (upper right), reactive oxygen species production (lower left), and the threshold for induction of the permeability transition (lower right). CsA treatment significantly improved all of these outcome measures. Bars are group means ± SEM, baseline indicates contralateral levels. Adapted from (Sullivan et al., 1999b).

Because CyP-D mRNA was found to be increased significantly in the spinal cord compared to that in cortex, and because of the lack of CsA neuroprotection in our SCI paradigm, we assessed CsA ability to inhibit mPT. The results clearly demonstrate that CsA was only partially effective in inhibiting Ca^{2+}-induced mPT in spinal cord mitochondria (Fig. 4). Bongkrekic acid, on the other hand, was highly effective in preventing Ca^{2+}-induced mPT maximum swelling in both cortical (15 ± 2.4%) and spinal cord (18 ± 3.6%) mitochondria (Sullivan et al., 2004).

Given the outcome of these studies, it could be argued that CsA dosage for SCI may need to be much higher than the optimal dosage used in TBI; however, given the toxicity of high doses of CsA, any beneficial effect could be offset. With this in mind, ongoing experiments in our laboratories are employing a nonimmunosuppressive CsA derivative, NIM811, that has been demonstrated to have minimal toxicity compared to CsA (Waldmeier et al., 2002) in both TBI and SCI models. The use of NIM811 will also test directly the hypothesis that the mPT is indeed pivotal to TBI/SCI-induced neu-

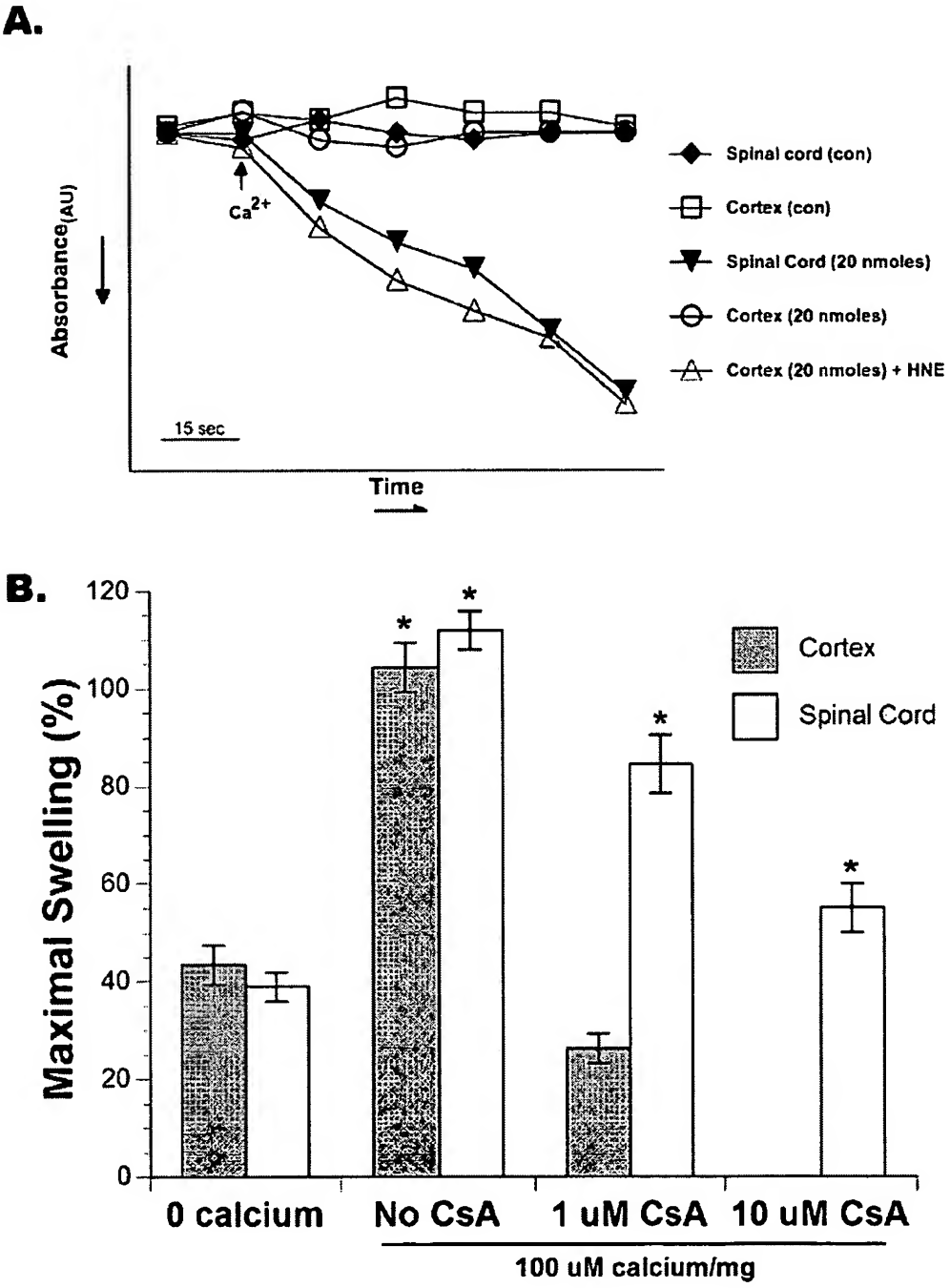

Fig. 4. Spinal cord mitochondria have a reduced threshold for Ca^{2+}-induced mitochondrial permeability transition (mPT) compared to that in cortical mitochondria isolated from normal, adult rats. Nonsynaptic mitochondria were isolated from the cortex and spinal cord and the induction of mPT was monitored by following light absorbance decreases, indicative of mitochondrial swelling (**A**). The addition of 20 nmol Ca^{2+}/mg of mitochondrial protein induced a rapid loss of absorbance in spinal cord but not cortical mitochondria. Preincubation of cortical mitochondria with the lipid peroxidation product 4-hydrononenonal (HNE; 10 μM) to increase oxidative stress altered their response Ca^{2+}. Not surprisingly, concentrations of Ca^{2+} greater than 100 nmol/mg (100 μM) induced CsA-sensitive swelling in either population of mitochondria (**B**). Mitochondrial swelling was expressed as a percentage of swelling measured in cortical or spinal cord mitochondria suspended in hypoosmotic buffer. CsA (1 μM) completely inhibited Ca^{2+}-induced swelling in cortical mitochondria but had little effect in spinal cord mitochondria. CsA (10 μM) was able to reduce Ca^{2+}-induced swelling in spinal cord mitochondria but did completely inhibit the mPT. Bars represent group means ± SEM. Adapted from Sullivan et al., 2004.

ronal cell death, and the underlying mechanism of CsA neuroprotection is not related to immunosuppression.

Our preliminary results indicate that NIM811 increases cortical tissue sparing, improves mitochondrial function, and reduces mitochondrial oxidative stress after TBI in adult rats. Unlike CsA, there seems to be no loss of neuroprotection associated with a higher dosage. NIM811 has also been shown to exert neuroprotection, as indicated by a reduction in oxidative damage and improved mitochondrial bioenergetics after SCI when administered at a significantly higher dosage than CsA. These results readily rule out neuroprotection mediated via calcineurin interactions and immunosuppression, but do not implicate completely or exclusively the mPT, because cyclophilins other than CyP-D could bind NIM811. Because no cyclophilins other than CyP-D have been ascribed any neuroprotective attributes, however, these results are overwhelming in support of mPT mod-

ulation in the neuroprotective effects of CsA and its derivatives after CNS injury.

CLOSING REMARKS

Strategies that target specific mitochondrial events may prove beneficial as therapeutic interventions after CNS injury. This is supported by a wealth of experimental data demonstrating that mitochondrial function is impaired severely after TBI and SCI and that this dysfunction is related to cell death pathways known to be activated in these distinct models. The loss of mitochondrial homeostasis that occurs after CNS injury implies that mPTP activation may be a common link in several models of TBI and SCI. The pathophysiologic role of mPT in CNS injury is also supported by several lines of scientific work that have utilized inhibitors (e.g., CsA and its derivatives) of the mPT in vivo to test this hypothesis. The development of new pharmacologic tools that specifically target the mPT and exhibit minimal toxicity will provide further support for its role in CNS injury and may prove beneficial as possible treatments for this and other neurodegenerative conditions.

REFERENCES

Azbill RD, Mu X, Bruce-Keller AJ, Mattson MP, Springer JE. 1997. Impaired mitochondrial function, oxidative stress and altered antioxidant enzyme activities following traumatic spinal cord injury. Brain Res 765: 283–290.

Baldwin SA, Fugaccia I, Brown DR, Brown LV, Scheff SW. 1996. Blood–brain barrier breach following cortical contusion in the rat. J Neurosurg 85:476–481.

Berden JH, Hoitsma AJ, Merx JL, Keyser A. 1985. Severe central-nervous-system toxicity associated with cyclosporin. Lancet 1:219–220.

Bernardi P. 1996. The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. Biochim Biophys Acta 1275:5–9.

Bernardi P, Broekemeier KM, Pfeiffer DR. 1994. Recent progress on regulation of the mitochondrial permeability transition pore; a cyclosporin-sensitive pore in the inner mitochondrial membrane. J Bioenerg Biomembr 26:509–517.

Beutner G, Ruck A, Riede B, Brdiczka D. 1998. Complexes between porin, hexokinase, mitochondrial creatine kinase and adenylate translocator display properties of the permeability transition pore. Implication for regulation of permeability transition by the kinases. Biochim Biophys Acta 1368:7–18.

Beutner G, Ruck A, Riede B, Welte W, Brdiczka D. 1996. Complexes between kinases, mitochondrial porin and adenylate translocator in rat brain resemble the permeability transition pore. FEBS Lett 396:189–195.

Bindokas VP, Lee CC, Colmers WF, Miller RJ. 1998. Changes in mitochondrial function resulting from synaptic activity in the rat hippocampal slice. J Neurosci 18:4570–4587.

Braughler JM, Hall ED. 1992. Involvement of lipid peroxidation in CNS injury. J Neurotrauma 9(Suppl):1–7.

Brenner C, Cadiou H, Vieira HL, Zamzami N, Marzo I, Xie Z, Leber B, Andrews D, Duclohier H, Reed JC, Kroemer G. 2000. Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator. Oncogene 19:329–336.

Broekemeier KM, Dempsey ME, Pfeiffer DR. 1989. Cyclosporin A is a potent inhibitor of the inner membrane permeability transition in liver mitochondria. J Biol Chem 264:7826–7830.

Brockemeier KM, Pfeiffer DR. 1995. Inhibition of the mitochondrial permeability transition by cyclosporin A during long time frame experiments: relationship between pore opening and the activity of mitochondrial phospholipases. Biochemistry 34:16440–16449.

Brustovetsky N, Brustovetsky T, Jemmerson R, Dubinsky JM. 2002. Calcium-induced cytochrome c release from CNS mitochondria is associated with the permeability transition and rupture of the outer membrane. J Neurochem 80:207–218.

Brustovetsky N, Brustovetsky T, Purl KJ, Capano M, Crompton M, Dubinsky JM. 2003. Increased susceptibility of striatal mitochondria to calcium-induced permeability transition. J Neurosci 23:4858–4867.

Brustovetsky N, Dubinsky JM. 2000. Dual responses of CNS mitochondria to elevated calcium. J Neurosci 20:103–113.

Budd SL, Nicholls DG. 1998. Mitochondria in the life and death of neurons. Essays Biochem 33:43–52.

Carriedo SG, Sensi SL, Yin HZ, Weiss JH. 2000. AMPA exposures induce mitochondrial Ca(2+) overload and ROS generation in spinal motor neurons in vitro. J Neurosci 20:240–250.

Clarke SJ, McStay GP, Halestrap AP. 2002. Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. J Biol Chem 277:34793–34799.

Connern CP, Halestrap AP. 1994. Recruitment of mitochondrial cyclophilin to the mitochondrial inner membrane under conditions of oxidative stress that enhance the opening of a calcium-sensitive non-specific channel. Biochem J 302:321–324.

Coyle JT, Puttfarcken P. 1993. Oxidative stress, glutamate, and neuronal degenerative disorders. Science 262:689–695.

de Groen PC, Aksamit AJ, Rakela J, Forbes GS, Krom RA. 1987. Central nervous system toxicity after liver transplantation. The role of cyclosporine and cholesterol. N Engl J Med 317:861–866.

Dugan LL, Sensi SL, Canzoniero LM, Handran SD, Rothman SM, Lin TS, Goldberg MP, Choi DW. 1995. Mitochondrial production of reactive oxygen species in cortical neurons following exposure to *N*-methyl-D-aspartate. J Neurosci 15:6377–6388.

Faden AI, Demediuk P, Panter SS, Vink R. 1989. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science 244:798–800.

Famiglio L, Racusen L, Fivush B, Solez K, Fisher R. 1989. Central nervous system toxicity of cyclosporine in a rat model. Transplantation 48:316–321.

Fiskum G. 2000. Mitochondrial participation in ischemic and traumatic neural cell death. J Neurotrauma 17:843–855.

Friberg H, Connern C, Halestrap AP, Wieloch T. 1999. Differences in the activation of the mitochondrial permeability transition among brain regions in the rat correlate with selective vulnerability. J Neurochem 72:2488–2497.

Friberg H, Wieloch T. 2002. Mitochondrial permeability transition in acute neurodegeneration. Biochimie 84:241–250.

Gebicki JM, Hunter FE Jr. 1964. Determination of swelling and disintegration of mitochondria with an electronic particle counter. J Biol Chem 239:631–639.

Halestrap AP, Brennerb C. 2003. The adenine nucleotide translocase: a central component of the mitochondrial permeability transition pore and key player in cell death. Curr Med Chem 10:1507–1525.

Halestrap AP, Davidson AM. 1990. Inhibition of Ca2(+)-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase. Biochem J 268:153–160.

Halestrap AP, McStay GP, Clarke SJ. 2002. The permeability transition pore complex: another view. Biochimie 84:153–166.

Halestrap AP, Woodfield KY, Connern CP. 1997. Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. J Biol Chem 272:3346–3354.

Hall ED, Braughler JM, McCall JM. 1992. Antioxidant effects in brain and spinal cord injury. J Neurotrauma 9(Suppl):165–172.

Hansson MJ, Mansson R, Mattiasson G, Ohlsson J, Karlsson J, Keep MF, Elmer E. 2004. Brain-derived respiring mitochondria exhibit homogeneous, complete and cyclosporin-sensitive permeability transition. J Neurochem 89:715–729.

Hunter FE Jr, Scott A, Hoffsten PE, Gebicki JM, Weinstein J, Schneider A. 1964a. Studies on the mechanism of swelling, lysis, and disintegration of isolated liver mitochondria exposed to mixtures of oxidized and reduced glutathione. J Biol Chem 239:614–621.

Hunter FE Jr, Scott A, Weinstein J, Schneider A. 1964b. Effects of phosphate, arsenate, and other substances on swelling and lipid peroxide formation when mitochondria are treated with oxidized and reduced glutathione. J Biol Chem 239:622–630.

Ichas F, Mazat JP. 1998. From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high- conductance state. Biochim Biophys Acta 1366:33–50.

Jiang D, Sullivan PG, Sensi SL, Steward O, Weiss JH. 2001. Zn(2+) induces permeability transition pore opening and release of pro-apoptotic peptides from neuronal mitochondria. J Biol Chem 276:47524–47529.

Kahan BD. 1989. Cyclosporine. N Engl J Med 321:1725–1738.

Kamal A, Ramakers GM, Urban IJ, De Graan PN, Gispen WH. 1999. Chemical LTD in the CA1 field of the hippocampus from young and mature rats. Eur J Neurosci 11:3512–3516.

Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen HC, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP. 1998. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J Neurosci 18: 687–697.

Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC. 2004. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature 427:461–465.

Lewen A, Fujimura M, Sugawara T, Matz P, Copin JC, Chan PH. 2001. Oxidative stress-dependent release of mitochondrial cytochrome c after traumatic brain injury. J Cereb Blood Flow Metab 21:914–920.

Liang LP, Ho YS, Patel M. 2000. Mitochondrial superoxide production in kainate-induced hippocampal damage. Neuroscience 101:563–570.

Lifshitz J, Friberg H, Neumar RW, Raghupathi R, Welsh FA, Janmey P, Saatman KE, Wieloch T, Grady MS, McIntosh TK. 2003. Structural and functional damage sustained by mitochondria after traumatic brain injury in the rat: evidence for differentially sensitive populations in the cortex and hippocampus. J Cereb Blood Flow Metab 23:219–231.

Lu YF, Tomizawa K, Moriwaki A, Hayashi Y, Tokuda M, Itano T, Hatase O, Matsui H. 1996. Calcineurin inhibitors, FK506 and cyclosporin A, suppress the NMDA receptor-mediated potentials and LTP, but not depotentiation in the rat hippocampus. Brain Res 729:142–146.

Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, Prevost MC, Xie Z, Matsuyama S, Reed JC, Kroemer G. 1998. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science 281:2027–2031.

McStay GP, Clarke SJ, Halestrap AP. 2002. Role of critical thiol groups on the matrix surface of the adenine nucleotide translocase in the mechanism of the mitochondrial permeability transition pore. Biochem J 367:541–548.

Nicholls DG, Budd SL. 2000. Mitochondria and neuronal survival. Physiol Rev 80:315–360.

Nicolli A, Basso E, Petronilli V, Wenger RM, Bernardi P. 1996. Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, a cyclosporin A-sensitive channel. J Biol Chem 271:2185–2192.

Okonkwo DO, Buki A, Siman R, Povlishock JT. 1999. Cyclosporin A limits calcium-induced axonal damage following traumatic brain injury. Neuroreport 10:353–358.

Okonkwo DO, Povlishock JT. 1999. An intrathecal bolus of cyclosporin A before injury preserves mitochondrial integrity and attenuates axonal disruption in traumatic brain injury. J Cereb Blood Flow Metab 19:443–451.

Prehn JH. 1998. Mitochondrial transmembrane potential and free radical production in excitotoxic neurodegeneration. Naunyn Schmiedebergs Arch Pharmacol 357:316–322.

Rabchevsky AG, Fugaccia I, Sullivan PG, Scheff SW. 2001. Cyclosporin A treatment following spinal cord injury to the rat: behavioral effects and stereological assessment of tissue sparing. J Neurotrauma 18:513–522.

Reynolds IJ, Hastings TG. 1995. Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. J Neurosci 15:3318–3327.

Rizzuto R, Bernardi P, Pozzan T. 2000. Mitochondria as all-round players of the calcium game. J Physiol 529:37–47.

Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, Pozzan T. 1999. Mitochondria as biosensors of calcium microdomains. Cell Calcium 26:193–199.

Scanlon JM, Reynolds IJ. 1998. Effects of oxidants and glutamate receptor activation on mitochondrial membrane potential in rat forebrain neurons. J Neurochem 71:2392–2400.

Scheff SW, Sullivan PG. 1999. Cyclosporin A significantly ameliorates cortical damage following experimental traumatic brain injury in rodents. J Neurotrauma 16:783–792.

Sengpiel B, Preis E, Krieglstein J, Prehn JH. 1998. NMDA-induced superoxide production and neurotoxicity in cultured rat hippocampal neurons: role of mitochondria. Eur J Neurosci 10:1903–1910.

Shiga Y, Onodera H, Matsuo Y, Kogure K. 1992. Cyclosporin A protects against ischemia-reperfusion injury in the brain. Brain Res 595:145–148.

Singleton RH, Stone JR, Okonkwo DO, Pellicane AJ, Povlishock JT. 2001. The immunophilin ligand FK506 attenuates axonal injury in an impact-acceleration model of traumatic brain injury. J Neurotrauma 18:607–614.

Springer JE, Azbill RD, Knapp PE. 1999. Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. Nat Med 5:943–946.

Stanton PK, Schanne FA. 1986. Hippocampal long-term potentiation increases mitochondrial calcium pump activity in rat. Brain Res 382:185–188.

Suehiro E, Singleton RH, Stone JR, Povlishock JT. 2001. The immunophilin ligand FK506 attenuates the axonal damage associated with rapid rewarming following posttraumatic hypothermia. Exp Neurol 172:199–210.

Sullivan PG, Bruce-Keller AJ, Rabchevsky AG, Christakos S, Clair DK, Mattson MP, Scheff SW. 1999a. Exacerbation of damage and altered NF-kappaB activation in mice lacking tumor necrosis factor receptors after traumatic brain injury. J Neurosci 19:6248–6256.

Sullivan PG, Geiger JD, Mattson MP, Scheff SW. 2000a. Dietary supplement creatine protects against traumatic brain injury. Ann Neurol 48: 723–729.

Sullivan PG, Keller JN, Bussen WL, Scheff SW. 2002. Cytochrome c release and caspase activation after traumatic brain injury. Brain Res 949:88–96.

Sullivan PG, Keller JN, Mattson MP, Scheff SW. 1998. Traumatic brain injury alters synaptic homeostasis: implications for impaired mitochondrial and transport function. J Neurotrauma 15:789–798.

Sullivan PG, Rabchevsky AG, Hicks RR, Gibson TR, Fletcher-Turner A, Scheff SW. 2000b. Dose-response curve and optimal dosing regimen of cyclosporin A after traumatic brain injury in rats. Neuroscience 101:289–295.

Sullivan PG, Rabchevsky AG, Keller JN, Lovell M, Sodhi A, Hart RP, Scheff SW. 2004. Intrinsic differences in brain and spinal cord mitochondria: implication for therapeutic interventions. J Comp Neurol 474:524–434.

Sullivan PG, Thompson M, Scheff SW. 2000c. Continuous infusion of cyclosporin A postinjury significantly ameliorates cortical damage following traumatic brain injury. Exp Neurol 161:631–637.

Sullivan PG, Thompson MB, Scheff SW. 1999b. Cyclosporin A attenuates acute mitochondrial dysfunction following traumatic brain injury. Exp Neurol 160:226–234.

Szabo I, Bernardi P, Zoratti M. 1992. Modulation of the mitochondrial megachannel by divalent cations and protons. J Biol Chem 267:2940–2946.

Szabo I, Zoratti M. 1991. The giant channel of the inner mitochondrial membrane is inhibited by cyclosporin A. J Biol Chem 266:3376–3379.

Szabo I, Zoratti M. 1992. The mitochondrial megachannel is the permeability transition pore. J Bioenerg Biomembr 24:111–117.

Uchino H, Elmer E, Uchino K, Li PA, He QP, Smith ML, Siesjo BK. 1998. Amelioration by cyclosporin A of brain damage in transient forebrain ischemia in the rat. Brain Res 812:216–226.

Uchino H, Elmer E, Uchino K, Lindvall O, Siesjo BK. 1995. Cyclosporin A dramatically ameliorates CA1 hippocampal damage following transient forebrain ischaemia in the rat. Acta Physiol Scand 155:469–471.

Waldmeier PC, Feldtrauer JJ, Qian T, Lemasters JJ. 2002. Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. Mol Pharmacol 62:22–29.

Waldmeier PC, Zimmermann K, Qian T, Tintelnot-Blomley M, Lemasters JJ. 2003. Cyclophilin D as a drug target. Curr Med Chem 10:1485–1506.

Walker RW, Brochstein JA. 1988. Neurologic complications of immunosuppressive agents. Neurol Clin 6:261–278.

Wieloch T. 2001. Mitochondrial involvement in acute neurodegeneration. IUBMB Life 52:247–254.

Woodfield K, Ruck A, Brdiczka D, Halestrap AP. 1998. Direct demonstration of a specific interaction between cyclophilin-D and the adenine nucleotide translocase confirms their role in the mitochondrial permeability transition. Biochem J 336:287–290.

Zoratti M, Szabo I. 1994. Electrophysiology of the inner mitochondrial membrane. J Bioenerg Biomembr 26:543–553.